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ENTITLED

METHOD OF REDUCING THE SENSITIVITY OF ASSAY DEVICES

BY

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METHOD OF REDUCING THE SENSITIVITY OF ASSAY DEVICES

Background of the Invention

Various analytical procedures and devices are commonly employed in flow-through assays to determine the presence and/or concentration of analytes that within a test sample. For instance, immunoassays utilize mechanisms of the immune systems, wherein antibodies are produced in response to the presence of antigens that are pathogenic or foreign to the organisms. These antibodies and antigens, i.e., immunoreactants, are capable of binding with one another, thereby causing a highly specific reaction mechanism that may be used to determine the presence or concentration of that particular antigen in a biological sample. Unfortunately, many conventional immunoassay assays encounter problems when used to measure test samples that possess a high analyte concentration. For instance, elevated levels of C-reactive protein ("CRP") are sometimes indicative of certain diseases, such as heart disease. However, even normal blood samples may contain a high CRP concentration, often within the "milligrams per milliliter" range. Because most conventional immunoassays are designed to detect analyte concentrations in the "nanograms per milliliter" range, they will almost undoubtedly give positive results of the presence of CRP for all test samples, including those having a normal CRP concentration. This may be particularly troubling to consumers who are themselves performing the assay with a disposable device.

As such, a need currently exists for an assay device that is capable of detecting the presence of an analyte in circumstances where a "normal" test sample still contains relatively high levels of the analyte.

Summary of the Invention

In accordance with one embodiment of the present invention, a method for detecting an analyte residing in a test sample is disclosed. The method comprises:

i) providing a flow-through assay device comprising a porous membrane that is in fluid communication with detection probes conjugated with a specific binding member for the analyte, wherein the assay device defines a scavenging zone and a detection zone, each of the zones containing a capture regent for the analyte;

ii) contacting the scavenging zone with the test sample so that a quantity of

the analyte less than or equal to a predefined base quantity binds to the capture reagent at the scavenging zone;

iii) contacting the conjugated detection probes with the test sample; and

iv) allowing the test sample and the conjugated detection probes to flow to the detection zone so that the conjugated detection probes or complexes thereof bind to the capture reagent and generate a detection signal, wherein the quantity of analyte in the test sample in excess of the predefined base quantity is proportional to the intensity of the detection signal.

In accordance with another embodiment of the present invention, a method for detecting an antigen residing in a test sample is disclosed. The method comprises:

i) providing a flow-through assay device comprising a porous membrane that is in fluid communication with detection probes conjugated with a specific binding member for the antigen, wherein the assay device defines a scavenging zone and a detection zone located downstream from the scavenging zone, each of the zones containing a capture reagent capable of specifically binding to the antigen, wherein the capture reagent of the scavenging zone includes an antibody;

ii) contacting the scavenging zone with the test sample so that a quantity of the antigen less than or equal to a predefined base quantity binds to the antibody at the scavenging zone;

iii) thereafter, contacting the conjugated detection probes with the test sample; and

iv) allowing the test sample and the conjugated detection probes to bind to the capture reagent at the detection zone and generate a detection signal, wherein the quantity of antigen in the test sample in excess of the predefined base quantity is proportional to the intensity of the detection signal.

In accordance with still another embodiment of the present invention, a flow-through assay device is disclosed for detecting an analyte residing in a test sample. The assay device comprises a porous membrane that is in fluid communication with detection probes conjugated with a specific binding member for the analyte. The assay device defines a scavenging zone that contains a capture reagent configured to bind to a quantity of the analyte less than or equal to a predefined base quantity. The assay device further defines a detection zone

within which a capture reagent is immobilized that is configured to bind to the conjugated detection probes or complexes of the conjugated detection probes and any analyte that does not bind to the scavenging zone. The detection zone is configured to generate a detection signal so that the quantity of analyte in the test sample in excess of the predefined base quantity is proportional to the intensity of the detection signal.

Other features and aspects of the present invention are discussed in greater detail below.

Brief Description of the Drawings

A full and enabling disclosure of the present invention, including the best mode thereof, directed to one of ordinary skill in the art, is set forth more particularly in the remainder of the specification, which makes reference to the appended figures in which:

Fig. 1 is a perspective view of one embodiment of a flow-through assay device of the present invention;

Fig. 2 is a graphical illustration of one embodiment for covalently conjugating an antibody to a detection probe;

Fig. 3 is a graphical illustration of the mechanism used for one embodiment of a sandwich assay format of the present invention; and

Fig. 4 shows the results of Example 2, in which the intensity of the detection line for an assay using a scavenging zone was compared to the intensity of the detection line for an assay without a scavenging zone.

Repeat use of reference characters in the present specification and drawings is intended to represent same or analogous features or elements of the invention.

Detailed Description of Representative Embodiments

Definitions

As used herein, the term "analyte" generally refers to a substance to be detected. For instance, analytes may include antigenic substances, haptens, antibodies, and combinations thereof. Analytes include, but are not limited to, toxins, organic compounds, proteins, peptides, microorganisms, amino acids, nucleic acids, hormones, steroids, vitamins, drugs (including those administered for therapeutic purposes as well as those administered for illicit purposes), drug

intermediaries or byproducts, bacteria, virus particles and metabolites of or antibodies to any of the above substances. Specific examples of some analytes include ferritin; creatinine kinase MB (CK-MB); digoxin; phenytoin; phenobarbitol; carbamazepine; vancomycin; gentamycin; theophylline; valproic acid; quinidine; luteinizing hormone (LH); follicle stimulating hormone (FSH); estradiol, progesterone; C-reactive protein; lipocalins; IgE antibodies; cytokines; vitamin B2 micro-globulin; glycated hemoglobin (Gly. Hb); cortisol; digitoxin; N-acetylprocainamide (NAPA); procainamide; antibodies to rubella, such as rubella-IgG and rubella IgM; antibodies to toxoplasmosis, such as toxoplasmosis IgG (Toxo-IgG) and toxoplasmosis IgM (Toxo-IgM); testosterone; salicylates; acetaminophen; hepatitis B virus surface antigen (HBsAg); antibodies to hepatitis B core antigen, such as anti-hepatitis B core antigen IgG and IgM (Anti-HBC); human immune deficiency virus 1 and 2 (HIV 1 and 2); human T-cell leukemia virus 1 and 2 (HTLV); hepatitis B e antigen (HBeAg); antibodies to hepatitis B e antigen (Anti-HBe); influenza virus; thyroid stimulating hormone (TSH); thyroxine (T4); total triiodothyronine (Total T3); free triiodothyronine (Free T3); carcinoembryoic antigen (CEA); lipoproteins, cholesterol, and triglycerides; and alpha fetoprotein (AFP). Drugs of abuse and controlled substances include, but are not intended to be limited to, amphetamine; methamphetamine; barbiturates, such as amobarbital, secobarbital, pentobarbital, phenobarbital, and barbital; benzodiazepines, such as librium and valium; cannabinoids, such as hashish and marijuana; cocaine; fentanyl; LSD; methaqualone; opiates, such as heroin, morphine, codeine, hydromorphone, hydrocodone, methadone, oxycodone, oxymorphone and opium; phencyclidine; and propoxyhene. Other potential analytes may be described in U.S. Patent Nos. 6,436,651 to Everhart, et al. and 4,366,241 to Tom et al.

As used herein, the term "test sample" generally refers to a material suspected of containing the analyte. The test sample may be used directly as obtained from the source or following a pretreatment to modify the character of the sample. The test sample may be derived from any biological source, such as a physiological fluid, including, blood, interstitial fluid, saliva, ocular lens fluid, cerebral spinal fluid, sweat, urine, milk, ascites fluid, mucous, synovial fluid, peritoneal fluid, vaginal fluid, amniotic fluid or the like. The test sample may be

pretreated prior to use, such as preparing plasma from blood, diluting viscous fluids, and the like. Methods of treatment may involve filtration, precipitation, dilution, distillation, mixing, concentration, inactivation of interfering components, and the addition of reagents. Besides physiological fluids, other liquid samples may be used such as water, food products and the like for the performance of environmental or food production assays. In addition, a solid material suspected of containing the analyte may be used as the test sample. In some instances it may be beneficial to modify a solid test sample to form a liquid medium or to release the analyte.

Detailed Description

Reference now will be made in detail to various embodiments of the invention, one or more examples of which are set forth below. Each example is provided by way of explanation of the invention, not limitation of the invention. In fact, it will be apparent to those skilled in the art that various modifications and variations may be made in the present invention without departing from the scope or spirit of the invention. For instance, features illustrated or described as part of one embodiment, may be used on another embodiment to yield a still further embodiment. Thus, it is intended that the present invention covers such modifications and variations as come within the scope of the appended claims and their equivalents.

In general, the present invention is directed to a flow-through assay device for detecting an analyte residing in a test sample. The device utilizes a scavenging zone that contains a capture reagent for the analyte of interest. The capture reagent may capture a quantity of the analyte that is less than or equal to a predefined base quantity of the analyte, such as a quantity considered "normal" for a particular test sample. Thus, the capture reagent is able to prevent some of the analyte from being detected. In this manner, the sensitivity of the assay device may be reduced in a simple, inexpensive, yet effective manner.

Referring to Fig. 1, for instance, one embodiment of a flow-through assay device 20 that may be formed according to the present invention will now be described in more detail. As shown, the device 20 contains a porous membrane 23 optionally supported by a rigid material 21. In general, the porous membrane 23 may be made from any of a variety of materials through which the test sample

is capable of passing. For example, the materials used to form the porous membrane 23 may include, but are not limited to, natural, synthetic, or naturally occurring materials that are synthetically modified, such as polysaccharides (e.g., cellulose materials such as paper and cellulose derivatives, such as cellulose acetate and nitrocellulose); polyether sulfone; polyethylene; nylon; polyvinylidene fluoride (PVDF); polyester; polypropylene; silica; inorganic materials, such as deactivated alumina, diatomaceous earth, $MgSO_4$, or other inorganic finely divided material uniformly dispersed in a porous polymer matrix, with polymers such as vinyl chloride, vinyl chloride-propylene copolymer, and vinyl chloride-vinyl acetate copolymer; cloth, both naturally occurring (e.g., cotton) and synthetic (e.g., nylon or rayon); porous gels, such as silica gel, agarose, dextran, and gelatin; polymeric films, such as polyacrylamide; and the like. In one particular embodiment, the porous membrane 23 is formed from nitrocellulose and/or polyether sulfone materials. It should be understood that the term "nitrocellulose" refers to nitric acid esters of cellulose, which may be nitrocellulose alone, or a mixed ester of nitric acid and other acids, such as aliphatic carboxylic acids having from 1 to 7 carbon atoms.

The device 20 may also contain a wicking pad 28. The wicking pad 28 generally receives fluid that has migrated through the entire porous membrane 23. As is well known in the art, the wicking pad 28 may assist in promoting capillary action and fluid flow through the membrane 23.

To initiate the detection of an analyte within the test sample, a user may apply the test sample to a portion of the porous membrane 23 (either directly or indirectly) through which it may then travel in the direction illustrated by arrow "L" in Fig. 1. For example, as shown, the test sample is applied to a sampling pad 17 that is in fluid communication with the porous membrane 23. Some suitable materials that may be used to form the sampling pad 17 include, but are not limited to, nitrocellulose, cellulose, porous polyethylene pads, and glass fiber filter paper.

The sampling pad 17 contains a scavenging zone 35. Alternatively, the scavenging zone 35 may be formed directly on the porous membrane 23, or at any other location of the assay device 20. Regardless of its location, the scavenging zone 35 includes one or more capture reagents having a specific binding member for the analyte of interest. A specific binding member generally refers to a member

of a specific binding pair, i.e., two different molecules where one of the molecules chemically and/or physically binds to the second molecule. For example, in some embodiments, the capture reagent may include antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, antibodies (e.g., polyclonal, monoclonal, etc.), and complexes thereof. When utilized, the antibody may be a monoclonal or polyclonal antibody, a recombinant protein or a mixture(s) or fragment(s) thereof, as well as a mixture of an antibody and other specific binding members. The details of the preparation of such antibodies and their suitability for use as specific binding members are well known to those skilled in the art. Other common specific binding pairs include but are not limited to, biotin and avidin (or derivatives thereof), biotin and streptavidin, carbohydrates and lectins, complementary nucleotide sequences (including probe and capture nucleic acid sequences used in DNA hybridization assays to detect a target nucleic acid sequence), complementary peptide sequences including those formed by recombinant methods, effector and receptor molecules, hormone and hormone binding protein, enzyme cofactors and enzymes, enzyme inhibitors and enzymes, and so forth. Furthermore, specific binding pairs may include members that are analogs of the original specific binding member. For example, a derivative or fragment of the analyte, i.e., an analyte-analog, may be used so long as it has at least one epitope in common with the analyte.

The capture reagent of the scavenging zone 35 serves as a binding site for the analyte. Specifically, analytes, such as antigens, typically have two or more binding sites (e.g., epitopes). One of these binding sites binds to the capture reagent at the scavenging zone 35. Desirably, the capture reagent is immobilized (diffusively or non-diffusively) within the scavenging zone 35 to prohibit the analyte from later being captured at the detection zone 31 (discussed below). Alternatively, however, the capture reagent may simply be formed from a compound that will not be captured at the detection zone 31. For example, the capture reagent may be identical to an additional capture reagent used at the detection zone 31. In this manner, the capture reagent at the scavenging zone 35 will occupy the only epitope of the analyte that would be able to bind to the capture reagent at the detection zone 31.

The scavenging zone 35 may generally provide any number of distinct

regions (e.g., lines, dots, etc.). Each region may contain the same capture reagent, or may contain different capture reagents for capturing multiple analytes. The regions may be disposed in the form of lines in a direction that is substantially perpendicular to the flow of the test sample through the assay device 20. Likewise, in some embodiments, the regions may be disposed in the form of lines in a direction that is substantially parallel to the flow of the test sample through the assay device 20. Regardless of the particular configuration of the scavenging zone 35 selected, the quantity of the capture reagent at the scavenging zone 35 is predetermined and tailored to capture a quantity of the analyte that is less than or equal to a predefined base quantity, such as a quantity considered "normal" for the particular application. For instance, a blood sample containing less than about 10 micrograms of C-reactive protein ("CRP") per milliliter may be considered "normal" under some circumstances. In this case, the quantity of CRP-specific antibodies utilized at the scavenging zone 35 may be sufficient to bind to a maximum of 10 micrograms per milliliter of CRP. Any additional CRP will pass through the scavenger zone 35 and be detected at the detection zone 31 (discussed below).

Referring again to Fig. 1, the test sample (including any free analyte) travels from the sampling pad 17 to a conjugate pad 22 that is in fluid communication with at least one end of the sampling pad 17. The conjugate pad 22 is formed from a material through which the test sample is capable of passing. For example, in one embodiment, the conjugate pad 22 is formed from glass fibers. Although only one conjugate pad 22 is shown, it should be understood that other conjugate pads may also be used in the present invention.

To facilitate accurate detection of the presence or absence of any remaining analyte within the test sample, a predetermined amount of detection probes are applied at various locations of the device 20. Any substance generally capable of generating a signal that is detectable visually or by an instrumental device may be used as detection probes. Various suitable substances may include chromogens; catalysts; luminescent compounds (e.g., fluorescent, phosphorescent, etc.); radioactive compounds; direct visual labels, including colloidal metallic (e.g., gold) and non-metallic particles, dye particles, enzymes or substrates, or organic polymer latex particles; liposomes or other vesicles containing signal producing substances; and so forth. For instance, some enzymes suitable for use as

detection probes are disclosed in U.S. Patent No. 4,275,149 to Litman, et al., which is incorporated herein in its entirety by reference thereto for all purposes. Some examples of suitable fluorescent molecules, for instance, include, but are not limited to, fluorescein, europium chelates, phycobiliprotein, rhodamine and their derivatives and analogs. Other suitable detection probes may be described in U.S. Patent Nos. 5,670,381 to Jou, et al. and 5,252,459 to Tarcha, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

The detection probes, such as described above, may be used alone or in conjunction with a particle (sometimes referred to as "beads"). For instance, naturally occurring particles, such as nuclei, mycoplasma, plasmids, plastids, mammalian cells (e.g., erythrocyte ghosts), unicellular microorganisms (e.g., bacteria), polysaccharides (e.g., agarose), and so forth, may be used. Further, synthetic particles may also be utilized. For example, in one embodiment, latex particles that are labeled with a fluorescent or colored dye are utilized. Although any latex particle may be used in the present invention, the latex particles are typically formed from polystyrene, butadiene styrenes, styreneacrylic-vinyl terpolymer, polymethylmethacrylate, polyethylmethacrylate, styrene-maleic anhydride copolymer, polyvinyl acetate, polyvinylpyridine, polydivinylbenzene, polybutyleneterephthalate, acrylonitrile, vinylchloride-acrylates, and so forth, or an aldehyde, carboxyl, amino, hydroxyl, or hydrazide derivative thereof. Other suitable particles may be described in U.S. Patent Nos. 5,670,381 to Jou, et al. and 5,252,459 to Tarcha, et al., which are incorporated herein in their entirety by reference thereto for all purposes. Commercially available examples of suitable fluorescent particles include fluorescent carboxylated microspheres sold by Molecular Probes, Inc. under the trade names “FluoSphere” (Red 580/605) and “TransfluoSphere” (543/620), as well as “Texas Red” and 5- and 6-carboxytetramethylrhodamine, which are also sold by Molecular Probes, Inc. In addition, commercially available examples of suitable colored, latex particles include carboxylated latex beads sold by Bang’s Laboratory, Inc.

When utilized, the shape of the particles may generally vary. In one particular embodiment, for instance, the particles are spherical in shape. However, it should be understood that other shapes are also contemplated by the present invention, such as plates, rods, discs, bars, tubes, irregular shapes, etc. In

addition, the size of the particles may also vary. For instance, the average size (e.g., diameter) of the particles may range from about 0.1 nanometers to about 1,000 microns, in some embodiments, from about 0.1 nanometers to about 100 microns, and in some embodiments, from about 1 nanometer to about 10 microns. For instance, "micron-scale" particles are often desired. When utilized, such "micron-scale" particles may have an average size of from about 1 micron to about 1,000 microns, in some embodiments from about 1 micron to about 100 microns, and in some embodiments, from about 1 micron to about 10 microns. Likewise, "nano-scale" particles may also be utilized. Such "nano-scale" particles may have an average size of from about 0.1 to about 10 nanometers, in some embodiments from about 0.1 to about 5 nanometers, and in some embodiments, from about 1 to about 5 nanometers.

In some instances, the detection probes are modified in some manner so that they are more readily able to bind to the analyte. In such instances, the detection probes may be modified with specific binding members that are adhered thereto to form conjugated probes. These specific binding members may be the same or different than the specific binding members described above. Specific binding members may generally be attached to detection probes using any of a variety of well-known techniques. For instance, covalent attachment of specific binding members to the detection probes (e.g., particles) may be accomplished using carboxylic, amino, aldehyde, bromoacetyl, iodoacetyl, thiol, epoxy and other reactive or linking functional groups, as well as residual free radicals and radical cations, through which a protein coupling reaction may be accomplished. A surface functional group may also be incorporated as a functionalized co-monomer because the surface of the detection probe may contain a relatively high surface concentration of polar groups. In addition, although detection probes are often functionalized after synthesis, in certain cases, such as poly(thiophenol), the particles are capable of direct covalent linking with a protein without the need for further modification. For example, referring to Fig. 2, one embodiment of the present invention for covalently conjugating a particle-containing detection probe is illustrated. As shown, the first step of conjugation is activation of carboxylic groups on the probe surface using carbodiimide. In the second step, the activated carboxylic acid groups are reacted with an amino group of an antibody to form an

amide bond. The activation and/or antibody coupling may occur in a buffer, such as phosphate-buffered saline (PBS) (e.g., pH of 7.2) or 2-(N-morpholino) ethane sulfonic acid (MES) (e.g., pH of 5.3). As shown, the resulting detection probes may then be blocked with ethanolamine, for instance, to block any remaining activated sites. Overall, this process forms a conjugated detection probe, where the antibody is covalently attached to the probe. Besides covalent bonding, other attachment techniques, such as physical adsorption, may also be utilized in the present invention.

Referring again to Fig. 1, the assay device 20 also contains a detection zone 31 within which is immobilized a capture reagent that is capable of binding to the conjugated detection probes. For example, in some embodiments, the capture reagent may be a biological capture reagent. Such biological capture reagents are well known in the art and may include, but are not limited to, antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, primary or secondary antibodies (e.g., polyclonal, monoclonal, etc.), and complexes thereof. The capture reagent serves as a stationary binding site for complexes formed between the analyte and conjugated detection probes. Upon reaching the detection zone 31, one of the binding sites of the analyte is occupied by the specific binding member of the conjugated probe. However, the free binding site of the analyte may bind to the immobilized capture reagent. Upon being bound to the immobilized capture reagent, the complexed probes form a new ternary sandwich complex.

The detection zone 31 may generally provide any number of distinct detection regions so that a user may better determine the concentration of a particular analyte within a test sample. Each region may contain the same capture reagents, or may contain different capture reagents for capturing multiple analytes. For example, the detection zone 31 may include two or more distinct detection regions (e.g., lines, dots, etc.). The detection regions may be disposed in the form of lines in a direction that is substantially perpendicular to the flow of the test sample through the assay device 20. Likewise, in some embodiments, the detection regions may be disposed in the form of lines in a direction that is substantially parallel to the flow of the test sample through the assay device.

Although the detection zone 31 may indicate the presence of an analyte, it

is often difficult to accurately determine the relative concentration of the analyte within the test sample under actual test conditions. Thus, the assay device 20 may also include a calibration zone 32. In this embodiment, the calibration zone 32 is formed on the porous membrane 23 and is positioned downstream from the detection zone 31. The calibration zone 32 is provided with a capture reagent that is capable of binding to probes, whether uncaptured detection probes or separate calibration probes, which pass through the detection zone 31. Similar to the detection zone 31, the calibration zone 32 may also provide any number of distinct calibration regions in any direction so that a user may better determine the concentration of a particular analyte within a test sample.

Each region may contain the same capture reagents, or may contain different capture reagents for capturing different types of probes. The capture reagents utilized in the calibration zone 32 may be the same or different than the capture reagents used in the detection zone 31. In one embodiment, the capture reagent for the calibration zone 32 is a polyelectrolyte, such as described in U.S. Publication No. 2003/0124739 to Song, et al., which is incorporated herein its entirety by reference thereto for all purposes. When utilized, the polyelectrolyte may have a net positive or negative charge, as well as a net charge that is generally neutral. For instance, some suitable examples of polyelectrolytes having a net positive charge include, but are not limited to, polylysine (commercially available from Sigma-Aldrich Chemical Co., Inc. of St. Louis, Missouri), polyethyleneimine; epichlorohydrin-functionalized polyamines and/or polyamidoamines, such as poly(dimethylamine-co-epichlorohydrin); polydiallyldimethyl-ammonium chloride; cationic cellulose derivatives, such as cellulose copolymers or cellulose derivatives grafted with a quaternary ammonium water-soluble monomer; and so forth. In one particular embodiment, CelQuat® SC-230M or H-100 (available from National Starch & Chemical, Inc.), which are cellulosic derivatives containing a quaternary ammonium water-soluble monomer, may be utilized. Moreover, some suitable examples of polyelectrolytes having a net negative charge include, but are not limited to, polyacrylic acids, such as poly(ethylene-co-methacrylic acid, sodium salt), and so forth. It should also be understood that other polyelectrolytes may also be utilized, such as amphiphilic polyelectrolytes (i.e., having polar and non-polar portions). For instance, some

examples of suitable amphiphilic polyelectrolytes include, but are not limited to, poly(styryl-b-N-methyl 2-vinyl pyridnium iodide) and poly(styryl-b-acrylic acid), both of which are available from Polymer Source, Inc. of Dorval, Canada.

Although any polyelectrolyte may generally be used, the polyelectrolyte selected for a particular application may vary depending on the nature of the detection probes, the porous membrane, and so forth. In particular, the distributed charge of a polyelectrolyte allows it to bind to substances having an opposite charge. Thus, for example, polyelectrolytes having a net positive charge are often better equipped to bind with probes that are negatively charged, while polyelectrolytes that have a net negative charge are often better equipped to bind to probes that are positively charged. Thus, in such instances, the ionic interaction between these molecules allows the required binding to occur within the calibration zone 32. Nevertheless, although ionic interaction is primarily utilized to achieve the desired binding in the calibration zone 32, it has also been discovered that polyelectrolytes may bind with detection probes having a similar charge.

The calibration regions may be pre-loaded on the porous membrane 23 with different amounts of the capture reagent so that a different signal intensity is generated by each calibration region upon migration of the probes. The overall amount of capture reagent within each calibration region may be varied by utilizing calibration regions of different sizes and/or by varying the concentration or volume of the capture reagent in each calibration region. If desired, an excess of detection probes may be employed in the assay device 20 so that each calibration region reaches its full and predetermined potential for signal intensity. That is, the amount of uncaptured detection probes that are deposited upon calibration regions are predetermined because the amount of the capture reagent employed on the calibration regions is set at a predetermined and known level. In the alternative, a predetermined amount of separate calibration probes may be used that are configured to only bind to the capture reagent at the calibration zone 32.

Referring to Fig. 3, one embodiment of a method for detecting the presence of an analyte will now be described in more detail. Initially, a test sample containing an analyte A is applied to the sampling pad 17. At the sampling pad 17, a certain quantity of the analyte A binds to a capture reagent 60 immobilized at the scavenging zone 35, such as an amount less than or equal to a predefined base

quantity of analyte considered "normal" for the particular test sample. From the sampling pad 17, any analyte A in excess of the predefined base quantity travels in the direction "L" to the conjugate pad 22, where it mixes with conjugated detection probes 41 and calibration probes 43 (may or may not be conjugated). In this embodiment, the excess analyte A binds with the conjugated detection probes 41 to form analyte/conjugated probe complexes 49. Because the scavenging zone 35 is positioned upstream from the conjugate pad 22, it is not necessary to supply detection probes 41 for binding to any of the analyte A that is already captured by the scavenging zone 35. In this manner, the overall amount of required probes is reduced, which provides substantial cost savings.

At the detection zone 31, the complexes 49 are captured by a capture reagent 90. If desired, the capture reagent 60 at the scavenging zone 35 is identical to the capture reagent 90. Thus, should any of the capture reagent 60 somehow become free from the scavenging zone 35 and travel to the detection zone 31, it will not bind to the capture reagent 90 and adversely impact the desired reduction in detection sensitivity. Further, the calibration probes 43 travel through the detection zone 31 to bind with a capture reagent (not shown) at the calibration zone 32.

Once captured, the signal of the probes at the detection zone 31 and calibration zone 32 may be measured using any known method of detection, such as visually or with a reading device. Regardless of the technique utilized, the quantity of the analyte in excess of the predefined base quantity may be ascertained by correlating the emitted signal, I_s, of the probes captured at the detection zone 31 to a predetermined analyte concentration. In some embodiments, the intensity signal, I_s, may also be compared with the emitted signal, I_c, of probes captured at the calibration zone 32. The total amount of the probes at the calibration zone 32 is predetermined and known and thus may be used for calibration purposes. For example, in some embodiments (e.g., sandwich assays), the quantity of analyte in excess of the predefined base quantity is directly proportional to the ratio of I_s to I_c. In other embodiments (e.g., competitive assays), the quantity of analyte in excess of the predefined base quantity is inversely proportional to the ratio of I_s to I_c. Based upon the intensity range in which the detection zone 31 falls, the general concentration range for the analyte may be

determined. As a result, calibration and sample testing may be conducted under approximately the same conditions at the same time, thus providing reliable quantitative or semi-quantitative results, with increased sensitivity.

If desired, the ratio of I_s to I_c may be plotted versus the analyte concentration for a range of known analyte concentrations to generate a calibration curve. To determine the quantity of analyte in an unknown test sample that is in excess of a predefined base quantity, the signal ratio may then be converted to analyte concentration according to the calibration curve. It should be noted that alternative mathematical relationships between I_s and I_c may be plotted versus the analyte concentration to generate the calibration curve. For example, in one embodiment, the value of $I_s /(I_s + I_c)$ may be plotted versus analyte concentration to generate the calibration curve.

Although various embodiments of device configurations have been described above, it should be understood, that a device of the present invention may generally have any configuration desired, and need not contain all of the components described above. Various other device configurations, for instance, are described in U.S. Patent Nos. 5,395,754 to Lambotte, et al.; 5,670,381 to Jou, et al.; and 6,194,220 to Malick, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

Various assay formats may also be used to test for the presence or absence of an analyte using the assay device of the present invention. For instance, in the embodiment described above, a "sandwich" format is utilized. Other examples of such sandwich-type assays are described in. by U.S. Patent Nos. 4,168,146 to Grubb, et al. and 4,366,241 to Tom, et al., which are incorporated herein in their entirety by reference thereto. An alternative technique is the "competitive-type" assay. In a "competitive-type" assay, the detection probe is typically a labeled analyte or analyte-analog that competes for binding of an antibody with any unlabeled analyte present in the sample. Competitive assays are sometimes used for detection of hapten analytes, each hapten being monovalent and capable of binding only one antibody molecule. Examples of competitive immunoassay devices are described in U.S. Patent Nos. 4,235,601 to Deutsch, et al., 4,442,204 to Liotta, and 5,208,535 to Buechler, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

The present inventor has discovered that a scavenging zone may be utilized to reduce the sensitivity of an assay device by capturing a quantity of an analyte within a test sample that is less than or equal to a predefined base quantity, such as a quantity that is considered "normal" for a particular test sample. In this manner, the analyte captured at the scavenging zone is not detected. Desirably, the test sample contacts the scavenging zone before mixing with detection probes minimize the required amount of detection probes and achieve substantial cost savings.

The present invention may be better understood with reference to the following examples.

EXAMPLE 1

The ability to form a lateral flow assay device according to the present invention was demonstrated. A nitrocellulose porous membrane (HF 120 from Millipore, Inc.) having a length of approximately 30 centimeters was laminated onto supporting cards. Goldline™ (a polylysine solution obtained from British Biocell International) was stripped onto the membrane to form a calibration line. In addition, monoclonal antibody for C-reactive protein (Mab2) (A#5804, available from BiosPacific, concentration of 1 milligram per milliliter) was immobilized on the porous membrane to form a detection line. The membrane samples were then dried for 1 hour at a temperature of 37°C. A cellulosic fiber wicking pad (Millipore, Inc. Co.) was attached to one end of the membrane. 120 microliters of gold particles conjugated with C-reactive protein (Mab1) (A#5811, available from BiosPacific, Inc.) was mixed with 250 microliters of sucrose in 630 microliters of water. The suspension was then loaded onto a 20-centimeter long glass fiber conjugate pad (Millipore Co.). The glass fiber pad was then dried at 37°C overnight and laminated to the supporting card. The nitrocellulose membrane was attached to one end of the conjugate pad, while a sample pad was attached to the other end.

Various types of sample pads were evaluated. Specifically, the sample pads tested were a nylon membrane available from Millipore, a nitrocellulose membrane available from Schleicher & Schuell, and a nitrocellulose membrane available from Millipore. A scavenging antibody, i.e., monoclonal antibody for C-reactive protein (Mab2) (A#5804, available from BiosPacific Inc.), was applied to

the sampling pads at various concentrations to form a scavenging zone. Specifically, the scavenging antibody was applied to the nylon membrane at concentrations of 0 and 0.5 milligrams per milliliter, to the Schleicher & Schuell membranes at a concentration of 0.5 milligrams per milliliter, and to the Millipore membrane at a concentration of 2.36 milligrams per milliliter. Each membrane was then dried at 37°C, cut into 4-millimeter strips, and put onto the conjugate pad to complete the assembly of a 4-millimeter full strip.

80 microliters of a C-reactive protein solution (170 nanograms per milliliter) was applied to each sampling pad. Each assay was visually observed for the detection signal intensity. For the nylon membrane sample pad, the detection signal intensity was the same for scavenging antibody concentrations of 0 and 0.5 milligrams per milliliter. For the Schleicher & Schuell nitrocellulose membrane, the detection signal was detected, but its intensity was reduced. For the Millipore nitrocellulose sample pad, the detection line gave no signal.

EXAMPLE 2

A lateral flow assay device was formed as described above in Example 1, except that the sample pad was made from a membrane containing ultra-high molecular weight polyethylene spherical particles, which was obtained from the Porex Corporation of Fairburn, Georgia under the name "Lateral-Flo" membrane. In addition, the concentration of the scavenging antibody was 1 milligram per milliliter. To this sample pad, a solution containing 40 microliters of 2% Tween 20 and 40 microliters of C-reactive protein was applied. The C-reactive protein concentrations tested were 0, 50 and 500 nanograms per milliliter. Comparative samples were also tested that did not contain the scavenging antibody. The assay was allowed to develop for approximately 10 minutes, and the detection line intensity was then read using a reflectance reader.

For the samples containing the scavenging antibody, the detection line was negative for C-reactive protein concentrations of 0 and 50 nanograms per milliliter. For the comparative sample, a C-reactive protein concentration of 50 nanograms per milliliter resulted in a strong positive signal for the detection line. The results are shown in Fig. 4.

EXAMPLE 3

A lateral flow assay device was formed as described above in Example 1,

except that the scavenging antibody was monoclonal antibody for C-reactive protein (Mab1) (A#5811, available from BiosPacific) at a concentration of 5.7 milligrams per milliliter. To the sampling pad, 1 microliter of various C-reactive protein solutions (CRP concentrations of 1000, 10000 and 50000 nanograms per milliliter) were applied and run with 150 microliters of diluent (Tween:PBS 10:6). The assay was allowed to develop for approximately 10 minutes. The detection signal intensity was visually observed. The device gave a positive result for a C-reactive protein concentration of 50 nanograms per milliliter, but a negative for C-reactive protein concentrations of 1 and 10 nanograms per milliliter.

While the invention has been described in detail with respect to the specific embodiments thereof, it will be appreciated that those skilled in the art, upon attaining an understanding of the foregoing, may readily conceive of alterations to, variations of, and equivalents to these embodiments. Accordingly, the scope of the present invention should be assessed as that of the appended claims and any equivalents thereto.